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A1 <120> Species Specific Identification of Spore-Producing
Microbes Using the Gene Sequence of Small Acid Soluble
Spore Coat Proteins for Amplification Based Diagnostics

<130> 0014939-001600US

<140> US 10/067,613

<141> 2002-02-04

<150> US 60/138,167

<151> 1999-06-08

<150> US 60/192,206

<151> 2000-03-27

<150> US 09/590,759

<151> 2000-06-08

<160> 38

<170> PatentIn Ver. 2.1

<210> 1

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Bacillus cereus
primer Bcsasp-B 5' designed for heterologous PCR
and sequenceing of Bacillus anthracis

<400> 1

atgagtaaaa aacaacaagg ttat

24

<210> 2

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Bacillus cereus
primer Bcsasp-B 3' designed for heterologous PCR
and sequenceing of Bacillus anthracis

<400> 2

ctgatttgag ctagaagatt gtga

24

<210> 3
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus cereus
primer Bcsasp-1 5' designed for heterologous PCR
and sequenceing of Bacillus anthracis

<400> 3
atgggaaaaa ataatagtgg aagt

24

<210> 4
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus cereus
primer Bcsasp-1 3' designed for heterologous PCR
and sequenceing of Bacillus anthracis

<400> 4
gcgggttagct ctaccaccaa gt

22

<210> 5
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus cereus
primer Bcsasp-2 5' designed for heterologous PCR
and sequenceing of Bacillus anthracis

<400> 5
atgtcagcta gcacaaataa att

23

<210> 6
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus cereus
primer Bcsasp-2 3' designed for heterologous PCR
and sequenceing of Bacillus anthracis

<400> 6
ttatttttgg taaccgccta a

21

<210> 7
<211> 161
<212> DNA
<213> Bacillus cereus

<220>
 <223> *Bacillus cereus* small acid-soluble spore protein 1
 (sasp-1) sequence

<400> 7
 cgtaatgaag tattagttcg aggcgctgaa caagctcttg atcaaatgaa atatgaaatt 60
 gcacaagagt ttggtgtaca acttggtgca gatacaacag ctcgttcaaa cggatctggt 120
 ggtggtgaaa ttacaaaacg tttagtagca atggcagaac a 161

<210> 8
 <211> 161
 <212> DNA
 <213> *Bacillus anthracis*

<220>
 <223> *Bacillus anthracis* small acid-soluble spore
 protein 1 (sasp-1) sequence

<400> 8
 cgtaatgaat tattagttcg aggtgctgaa caagctcttg atcaaatgaa atatgaaatt 60
 gcacaagagt ttggtgtaca acttggtgca gatacaacag ctcgttcaaa tggatctggt 120
 ggtggtgaaa ttacaaaacg tttagtagca atggctgaac a 161

<210> 9
 <211> 154
 <212> DNA
 <213> *Bacillus anthracis* and *Bacillus cereus*

<220>
 <223> *Bacillus anthracis* and *Bacillus cereus* small
 acid-soluble spore protein 2 (sasp-2) sequence

<400> 9
 agcggttcct ggtgctgaat cagcattaga ccaaataaaa tacgaaatcg ctcaagagtt 60
 tgggtgtcaa cttggagctg atgcaacagc tcgcgctaac gggtctggtg gtggcgaaat 120
 cactaaacgt ctagtttcac tagctgagca acaa 154

<210> 10
 <211> 231
 <212> DNA
 <213> *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus* sp.

<220>
 <223> *Bacillus cereus* Bcer3 and BcerPub, *Bacillus thuringiensis* BtT,
 BtU, BtS, BtR, BtO, BtJ, 4J2, BtG and BtI and unidentified
Bacillus 1B/A small acid-soluble spore protein B (sasp-B)
 sequence

<400> 10
 aacaaagcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
 tcaactgaaa cagatgtaca agctgtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
 gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
 acagacgtgc attctgtgaa aaaacaaaat gctaagtcag ctgcaaaaaca a 231

<210> 11
<211> 237
<212> DNA
<213> Bacillus anthracis

<220>
<223> Bacillus anthracis small acid-soluble spore
protein B (sasp-B) sequence, amplicon from 38
Bacillus anthracis strains

<400> 11
aacaaggcaa cttctggtgc tagcattcaa agcacaaatg ctagttatgg tacagagttt 60
gcgactgaaa caaatgtaca agcagtaaaa caagcaaacg cacaatcaga agctaagaaa 120
gcgcaagctt ctggtgctag cattcaaagc acaaatgcta gttatggtag agaatttgca 180
actgaaacag acgtgcatgc tgtgaaaaaa caaatgcac aatcagctgc aaaacaa 237

<210> 12
<211> 231
<212> DNA
<213> Bacillus anthracis and Bacillus sp.

<220>
<223> Bacillus anthracis NMRI#15 and unidentified Bacillus 1B, 003,
III and IV small acid-soluble spore protein B (sasp-B) sequence

<400> 12
aacaaggcaa cttctggcgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
tcaactgaaa cagatgtgca agcagtaaaa caagcaaatg cacaatcaga agcaaagaaa 120
gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtactgaatt tgcaactgaa 180
acagatgtgc atgctgtgaa aaaacaaaat gcacaatcag ctgcaaaaaca a 231

<210> 13
<211> 231
<212> DNA
<213> Bacillus thuringiensis

<220>
<223> Bacillus thuringiensis BtB small acid-soluble spore protein B
(sasp-B) sequence

<400> 13
aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
tcaactgaaa cagatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtactgaatt tgcaactgaa 180
acagacgtgc atgctgtgaa aaaacaaaat gcacaatcag ctgcaaaaaca a 231

<210> 14
<211> 231
<212> DNA
<213> Bacillus thuringiensis

<220>
<223> Bacillus thuringiensis BtY small acid-soluble spore protein B
(sasp-B) sequence

<400> 14
aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
tcaactgaaa cagatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120

gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
 acagacgtgc atgctgtgaa aaaacaaaat gcacaatcag ctgcaaaaca a 231

<210> 15
 <211> 231
 <212> DNA
 <213> *Bacillus thuringiensis*

<220>
 <223> *Bacillus thuringiensis* 4A1, BtV and BtZ small acid-soluble
 spore protein B (sasp-B) sequence

<400> 15
 aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
 gcaactgaaa caaatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
 gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
 acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaca a 231

<210> 16
 <211> 231
 <212> DNA
 <213> *Bacillus thuringiensis*

<220>
 <223> *Bacillus thuringiensis* BtL small acid-soluble spore protein B
 (sasp-B) sequence

<400> 16
 aacaaagcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
 tcaactgaaa cagatgtaca agctgtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
 gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
 acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaca a 231

<210> 17
 <211> 231
 <212> DNA
 <213> *Bacillus cereus*

<220>
 <223> *Bacillus cereus* Bcer2 small acid-soluble spore protein B
 (sasp-B) sequence

<400> 17
 aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
 tcaactgaaa cagatgtaca agctgtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
 gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
 acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaca a 231

<210> 18
 <211> 231
 <212> DNA
 <213> *Bacillus thuringiensis*

<220>
 <223> *Bacillus thuringiensis* BtC, BtE2, BtE4, BtK, BtM and BtN small
 acid-soluble spore protein B (sasp-B) sequence

<400> 18
aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
gcgactgaaa caaatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaaca a 231

<210> 19
<211> 231
<212> DNA
<213> Bacillus thuringiensis and Bacillus cereus

<220>
<223> Bacillus thuringiensis BtP and BtZ and Bacillus cereus Bcer1
small acid-soluble spore protein B (sasp-B) sequence

<400> 19
aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagaattht 60
gctactgaaa caaatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaaca a 231

<210> 20
<211> 231
<212> DNA
<213> Bacillus thuringiensis

<220>
<223> Bacillus thuringiensis BtQ and BtW small acid-soluble spore
protein B (sasp-B) sequence

<400> 20
aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
gcgactgaaa caaatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
gcacaagctt ctggtgcaca aagtgcaaac gctagttatg gtacagaatt tgcaactgaa 180
acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaaca a 231

<210> 21
<211> 231
<212> DNA
<213> Bacillus anthracis

<220>
<223> Bacillus anthracis NMRI#57 small acid-soluble spore protein B
(sasp-B) sequence

<400> 21
aacaaggcaa cttctggtgc tagcattcaa agtacaaatg ctagttatgg tacagagttt 60
gcgactgaaa caaatgtaca agcagtaaaa caagcaaacg cacaatcaga agcaaagaaa 120
gcacaagctt ctggtgcaca aagtgcaaac gctagctatg gtacagaatt tgcaactgaa 180
acagacgtgc atgctgtgaa aaaacaaaat gctaagtcag ctgcaaaaaca a 231

<210> 22
<211> 355
<212> DNA
<213> Bacillus subtilis

<220>
 <223> Bacillus subtilis small acid-soluble spore protein
 gamma (sasp-gamma) sequence

<400> 22
 atttctaattg tcgtggaggt gataaacatg gctaactcaa acaacaaaac aaacgctcaa 60
 caagtaagaa aacaaaacca acaatcagca tctggccaag gtcagtttgg tacagaattt 120
 gctagcgaaa caaacgtaca acaagtaaga aaacaaaacc aacaatcagc tgctggacaa 180
 ggacaattcg gcactgaatt cgctagtga actgatgctc agcaagtaag acagcaaaac 240
 caatctgctg aacaaaacaa acaacaaaac agctaatac tgaaacagaa aaaaagcact 300
 tcatcctcgg gtggaagtgc ttttttcttt ttataaaacg acaaaacttg tggaa 355

<210> 23
 <211> 384
 <212> DNA
 <213> Bacillus globigii

<220>
 <223> Bacillus globigii small acid-soluble spore protein
 gamma (sasp-gamma) sequence

<400> 23
 ttgacgcgga cgctcactgc tcgttttaaaa attttttaaaa aagaggaata gctatacgat 60
 cacctgcaca ttctaatacgc cgtggaggtg ataacaatgg ctaactcaaa taacttcagc 120
 aaaacaaaacg ctcaacaagt tagaaaacaa aaccaacaat cagctgctgg tcaaggtcaa 180
 tttggcactg aatttgctag cgaaacaaac gctcagcaag tcagaaaaca aaaccagcaa 240
 tcagctggac aacaaggtca attcggcact gaattcgcta gtgaaactga cgcacagcag 300
 gtaagacagc aaaaccaatc tgctgaacaa aacaaacaac aaaacagcta atcactgaaa 360
 cagaaaaaag cacttcactc tcgg 384

<210> 24
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Bacillus
 globigii sasp-gamma forward primer BgSaspGam 5'

<400> 24
 acatggctaa ctcaacaac aa 22

<210> 25
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Bacillus
 globigii sasp-gamma reverse primer BgSaspGam 3'

<400> 25
 ggttttgttt tcttacttgt tgtac 25

<210> 26
 <211> 7
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:unique Bacillus anthracis sasp-B insertion, insertion region distinguishing Bacillus anthracis from other Bacillus species

<400> 26
 tagcatt

7

<210> 27
 <211> 77
 <212> PRT
 <213> Bacillus thuringiensis and Bacillus cereus

<220>
 <223> Bacillus thuringiensis 4D4 and Bacillus cereus Bcerp small acid-soluble spore protein B (sasp-B) sequence

<400> 27
 Asn Lys Ala Thr Ser Gly Ala Ser Ile Gln Ser Thr Asn Ala Ser Tyr
 1 5 10 15
 Gly Thr Glu Phe Ser Thr Glu Thr Asp Val Gln Ala Val Lys Gln Ala
 20 25 30
 Asn Ala Gln Ser Glu Ala Lys Lys Ala Gln Ala Ser Gly Ala Gln Ser
 35 40 45
 Ala Asn Ala Ser Tyr Gly Thr Glu Phe Ala Thr Glu Thr Asp Val His
 50 55 60
 Ser Val Lys Lys Gln Asn Ala Lys Ser Ala Ala Lys Gln
 65 70 75

<210> 28
 <211> 77
 <212> PRT
 <213> Bacillus thuringiensis

<220>
 <223> Bacillus thuringiensis BtK small acid-soluble spore protein B (sasp-B) sequence

<400> 28
 Asn Lys Ala Thr Ser Gly Ala Ser Ile Gln Ser Thr Asn Ala Ser Tyr
 1 5 10 15
 Gly Thr Glu Phe Ala Thr Glu Thr Asn Val Gln Ala Val Lys Gln Ala
 20 25 30
 Asn Ala Gln Ser Glu Ala Lys Lys Ala Gln Ala Ser Gly Ala Gln Ser
 35 40 45
 Ala Asn Ala Ser Tyr Gly Thr Glu Phe Ala Thr Glu Thr Asp Val His
 50 55 60
 Ala Val Lys Lys Gln Asn Ala Lys Ser Ala Ala Lys Gln
 65 70 75

<210> 29
 <211> 77
 <212> PRT
 <213> Bacillus thuringiensis

<220>
 <223> Bacillus thuringiensis BtB small acid-soluble spore
 protein B (sasp-B) sequence

<400> 29
 Asn Lys Ala Thr Ser Gly Ala Ser Ile Gln Ser Thr Asn Ala Ser Tyr
 1 5 10 15
 Gly Thr Glu Phe Ser Thr Glu Thr Asp Val Gln Ala Val Lys Gln Ala
 20 25 30
 Asn Ala Gln Ser Glu Ala Lys Lys Ala Gln Ala Ser Gly Ala Gln Ser
 35 40 45
 Ala Asn Ala Ser Tyr Gly Thr Glu Phe Ala Thr Glu Thr Asp Val His
 50 55 60
 Ala Val Lys Lys Gln Asn Ala Gln Ser Ala Ala Lys Gln
 65 70 75

<210> 30
 <211> 79
 <212> PRT
 <213> Bacillus anthracis

<220>
 <223> Bacillus anthracis small acid-soluble spore protein B
 (sasp-B) sequence

<400> 30
 Asn Lys Ala Thr Ser Gly Ala Ser Ile Gln Ser Thr Asn Ala Ser Tyr
 1 5 10 15
 Gly Thr Glu Phe Ala Thr Glu Thr Asn Val Gln Ala Val Lys Gln Ala
 20 25 30
 Asn Ala Gln Ser Glu Ala Lys Lys Ala Gln Ala Ser Gly Ala Ser Ile
 35 40 45
 Gln Ser Thr Asn Ala Ser Tyr Gly Thr Glu Phe Ala Thr Glu Thr Asp
 50 55 60
 Val His Ala Val Lys Lys Gln Asn Ala Gln Ser Ala Ala Lys Gln
 65 70 75

<210> 31
 <211> 76
 <212> PRT
 <213> Bacillus mycoides

<223> Bacillus mycoides small acid-soluble spore protein B
 (sasp-B) sequence

<400> 31
 Asn Lys Ala Thr Ser Gly Ala Ser Ile Gln Ser Thr Asn Ala Ser Tyr
 1 5 10 15
 Gly Thr Glu Phe Ala Thr Glu Thr Asn Val Gln Ala Val Lys Gln Ala
 20 25 30
 Asn Ala Gln Ser Glu Ala Gln Lys Ala Gln Ala Ser Ala Ala Gln Ser
 35 40 45
 Ala Asn Ala Ser Tyr Gly Thr Glu Phe Ala Thr Glu Thr Asp Val His
 50 55 60

Ala Val Lys Lys Gln Asn Ala Gln Ser Ala Ala Lys
65 70 75

<210> 32
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus anthracis BaSPB7 primer

<400> 32
gttatggtac agagtttgcg 20

<210> 33
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus anthracis BaSPB8 primer

<400> 33
ttgttttgca gctgattgt 19

<210> 34
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus anthracis oligonucleotide probe BaSPB2 (inverted, 'lower strand' sequence)

<400> 34
gcatttggtgc tttgaatgct a 21

<210> 35
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus anthracis oligonucleotide probe BaSPB4 (inverted, 'lower strand' sequence)

<400> 35
catttggtgct ttgaatgcta 20

<210> 36
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Bacillus
anthracis oligonucleotide probe BaSPB5 (direct,
'upper strand' sequence)

<400> 36
agcttctggt gctagcatt

19

<210> 37
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Clostridium
perfringens sasp-2 primer CPssp2-1

<400> 37
aataactaag gaggaatgaa aaatgt

26

<210> 38
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Clostridium
perfringens sasp-2 primer CPssp2-2

<400> 38
ttgttctacc attcttttaa ccatt

25